PB85-121002

Toxicological Data on Selected Hazardous Chemicals and Possible Extrapolation to Man

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Oct 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-121002

EPA-600/D-84-261
October 1984

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/D-84-261	2.
3. RECIPIENT'S ACCESSION NO. PB85 121002	
4. TITLE AND SUBTITLE Toxicological Data on Selected Hazardous Chemicals and Possible Extrapolation to Man	5. REPORT DATE October 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Richard J. Bull	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology & Microbiology Division, HERL U.S. Environmental Protection Agency, ORD Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. ABMC1A
	11. CONTRACT/GRANT NO. (in-house)
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The experimental evidence that chemicals commonly found in drinking water possess carcinogenic properties is reviewed. Three types of sources for chemicals in drinking water are examined; those which are contaminants of drinking water sources, those that arise as by-products of drinking water treatment, and those that arise in the distribution of drinking water. The evidence for carcinogenicity of chemicals that has been obtained from studies employing vegetable oil gavage for carbon tetrachloride, dichloroethylene, trichloroethylene, tetrachloroethylene and chloroform is critically examined. New evidence for the carcinogenicity of acrylamide is reviewed and compared to earlier evidence for epichlorohydrin carcinogenicity. Finally, the relative carcinogenic activity associated with coal tar- versus asphaltic-based paints used in water storage and distribution systems are compared.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 62
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

TOXICOLOGICAL DATA ON SELECTED HAZARDOUS CHEMICALS AND POSSIBLE EXTRAPOLATION TO MAN

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INTRODUCTION

A wide variety of chemicals have been found in drinking waters around the world. The presence of toxic chemicals in finished drinking water arises from three distinct sources:

1. Contamination of source water.
2. By-products of treatment processes.
3. Leaching from the distribution system.

The diversity of chemicals that have been found in drinking water using modern analytical techniques renders comprehensive review of toxic and potentially carcinogenic properties of each substance virtually impossible. In particular, the type of chemical contaminates found in drinking water sources is very dependent upon the nature of local industry, the type of water supply and regulations applied to industrial and municipal waste disposal practices. However, a number of chemicals do stand out as common contaminancs of drinking water by virtue of widespread use in commerce and industry and large production volumes.

Chemicals that are associated with drinking water treatment include disinfectants, coagulants, coagulant aids, oxidants and softening agents.

In recent years, it has become apparent that disinfectants, such as chlorine, are responsible for the formation of potentially significant quantities of chemicals in finished drinking water that possess carcinogenic and other toxic properties. Certain polymeric agents that are used in the clarification of drinking water are synthesized from monomeric components that possess similar undesireable properties. In both these cases, the nature of the chemicals produced and/or that remain in finished drinking water are still only partially understood. Nevertheless, there is direct evidence that a number of the chemicals that are associated with these processes do possess carcinogenic, mutagenic and other toxic properties.

Materials used in the construction and/or coating of storage tanks, water mains and pipe sometimes contain or are synthesized from chemicals that possess toxicological properties that are of potential concern. In most instances, the extent to which these materials are solubilized or otherwise present in drinking water at the tap has not been well documented. In some cases that involve particularly corrosive waters the concentrations of potentially dangerous chemicals in drinking water at the tap are substantially increased over levels found in the source water. The present review will be confined to two coatings considered as potential carcinogenic hazards.

The purpose the present paper is to review toxicological data that is available on the most common contaminants of drinking water. Particular attention will be focussed on those chemicals for which evidence of carcinogenic and mutagenic properties exist. The intent is to place the potential carcinogenic hazards associated with drinking water in perspective,

but with the full recognition that this is but one of serveral considerations which must be taken into account in producing a safe and wholesome drinking water. It should always be kept in mind that correcting perceived carcinogenic hazards may compromize other public health principles. Actions taken should only follow careful consideration of the hazards that may be associated with different corrective measures in a particular water supply.

CONTAMINATES OF SOURCE

A great variety of chemicals can contaminate sources of drinking water. By far the most frequent contaminates in this category are trichloroethylene and tetrachloroethylene because of their large production volumes and widespread use in commerce. Other chemicals that occur less frequently or at lower concentrations include arsenic, dichloromethane, carbon tetrachloride, chloroethylene, the three possible isomers of dichloroethylene and dichloroethane, 1,2-dibromoethane, 1,2-dibromo-3-chloropropane and benzene. A number of these chemicals are recognized human carcinogens (chloroethylene, benzene and arsenic) whose properties have been recently reviewed by IARC (1979a; 1982a; 1980; respectively). Other chemicals in this group either occur only under special circumstances so as to be primarily of local interest or are well accepted as carcinogens [1,2-dibromoethane (IARC, 1977), 1,2-dichloroethane (IARC, 1979]. On the other hand, the carcinogenicity of the dichloroethylenes, trichloroethylene, tetrachloroethylene and to a lesser degree carbon

tetrachloride have been somewhat controversial in recent years. Consequently, this section will provide an updated review of the data bearing on the carcinogenic properties of these chemicals and consideration of other contaminates will be for comparative purposes only.

In recent years an argument has developed within the scientific community that consideration of a chemical's carcinogenicity include evidence of direct interaction of a chemical or its metabolites with cellular DNA. This argument recognizes that a variety of mechanisms can contribute to an increased incidence of cancer including recurrent cellular toxicity and resulting cell replacement. In contrast to hypotheses suggesting the absence of thresholds with "genetoxic" carcinogens, cancer induced by such a mechanism would not be expected at doses below those producing overt cellular toxicity. The major, practical impact would be that such chemicals would be treated differently by regulatory agencies than those that act through a somatic cell mutation (Weisburger and Williams, 1983). Consequently, a major thrust of the present review will be to determine the extent to which the existence of data on this issue allows a more accurate projection of carcinogenic hazards to man. A summary of pertinent carcinogenicity and mutagenicity data associated with contaminants of source waters is provided in Tables 1 and 2.

CARBON TETRACHLORIDE

Studies of carbon tetrachloride (Cameron and Karunaratne, 1936; Reuber and Glover 1967a; Reuber and Glover 1967b) have documented the ability of carbon tetrachloride to induce preneoplastic changes in the liver of rats (e.g., hyperplastic nodules and cholangiofibrosis). Subsequent studies (Reuber and Glover, 1970) demonstrated that subcutan-

eous administration of carbon tetrachloride in corn oil at a dose of 2080 mg/kg twice weekly gave rise to hepatocellular carcinomas in male Japanese rats, Osborne-Mendel rats, and Wistar rats. Black rats and Sprague-Dawley rats failed to survive long enough to develop carcinomas following the same treatment. The authors' called attention to the fact that carcinoma development appeared to be inversely related to the degree of cirrhosis across the strains tested. However, this relationship may be fortuitous since those strains developing severe cirrhosis failed to survive. The single level of treatment utilized in this study precludes a clear resolution of this issue. A more recent study (NCI, 1976) of the carcinogenicity of carbon tetrachloride in the liver of the Osborne-Mendel rat failed to confirm its carcinogenic effects at doses of up to 160 mg CCl_4/kg in corn oil administered 5 times weekly for 78 weeks (47 and 94 mg/kg for males, 80 and 159 mg/kg for females). However, it should be noted that the doses utilized were much lower than used in previous studies.

Experiments by Della Porta (1961) indicated hepatoma development in male and female Syrian golden hamsters following treatment with 12.5 ul CCl_4 per week for 7 weeks and 6.25 ul for 23 additional weeks. However, there were no control animals included in this study making evaluation of the data difficult.

Eschenbrenner and Miller (1943) demonstrated that carbon tetrachloride was able to induce hepatomas in Strain A/J mice. Tumor yield was generally dose-related, but the effect was enhanced as the spacing between doses was increased from 1 to 5 day intervals. In subsequent experiments Eschenbrenner & Miller (1946) examined the relationship

between liver necrosis and tumor development and found that the liver became resistant to necrotizing doses of carbon tetrachloride with chronic treatment. This observation was most probably related to the inactivation of cytochrome P-450 by carbon tetrachloride observed in modern metabolic studies (Glende et al., 1975). Overall, there appeared to be a relationship between obvious necrosis and the development of hepatomas. However, hepatomas could be induced by doses of carbon tetrachloride that did not induce obvious necrosis in the liver. The authors pointed out that they could not rule out the possibility of a slightly greater rate of liver cell replacement at these lower doses. Kiplinger and Kensler (1963) also observed the induction of hepatomas following oral administration of carbon tetrachloride in C3H mice relative to historical controls, however, this study did not include concurrent controls. The National Cancer Institute (NCI, 1976) documented a high incidence of hepatocellular carcinomas in the B6C3F1 mice administered at doses of 1250 and 2500 mg/kg by gavage in a corn oil vehicle.

In summary, it is clear from the studies available that carbon tetrachloride is capable of inducing liver tumors at appropriate doses in either mice or rats and probably hamsters. Negative results with carbon tetrachloride have been confined to circumstances in which relatively low doses have been administered (e.g. the NCI rat study) or where a particular rat strain displayed such sensitivity to the hepatotoxic effects of carbon tetrachloride that they did not survive long enough to develop tumors (the Black and Sprague-Dawley rats in the Reuber and Glover study). Consequently, it must be concluded that carbon tetrachloride probably represents a carcinogenic risk to man.

It should be pointed out that there is little convincing evidence to indicate that carbon tetrachloride possesses genotoxic properties (IARC, 1979b; USEPA, 1984).

The ability of hepatonecrotic doses of carbon tetrachloride to promote the yield of liver tumors in mice is well documented (Pound and McGuire, 1975). More recent studies indicate that liver damage was detectable in both mice (Bruckner et al., 1984) and rats (Hayes et al., 1984) at daily doses of 10 and 12 mg/kg per day administered subchronically (90 days), respectively. Histopathological evidence of treatment-related focal neurosis was evident at these doses. Such doses are considerably below those that have been utilized in documenting the carcinogenic effects of carbon tetrachloride. Therefore, the bulk of the evidence indicates that the cancer producing activity of carbon tetrachloride are secondary to its hepatoxic effects. This should not be taken to mean that long-term repeated exposure to carbon tetrachloride does not represent a carcinogenic hazard.

DICHLOROETHYLENES:

All three isomers of dichloroethylene have been identified in drinking water. 1,1-Dichloroethylene (vinylidene chloride, 1,1-DCE) is the only isomer for which direct evidence exists relative to its carcinogenicity in experimental animals (IARC, 1979c). Maltoni et al. (1977) reported increased incidence of adenocarcinomas in the kidneys of male Swiss mice exposed to 25 ppm of 1,1-DCE in air 4 hours per day 4 to 5 days a week for 52 weeks at 98 weeks on study (final results have not yet been published). In a study that was terminated after only 12 months small increases in bronchiolo-alveolar adenomas and angiosarcomas in the liver

of CD-1 mice and angiosarcomas in 2 of 72 CD rats were observed with 1,1-DCE at levels of 55 ppm in air (Lee et al., 1978). Small increases in mammary fibroadenomas and carcinomas were observed after 82 weeks in Sprague-Dawley rats exposed to levels of up to 150 ppm in air for 4 hours/day for 4-5 days a week for 52 weeks (Maltoni et al., 1977).

More recent studies have failed to demonstrate carcinogenic responses to 1,1-DCE administration. The National Toxicology Program (USA) completed a study of 1,1-DCE carcinogenicity in Fischer 344 rats and B6C3F1 mice (NTP, 1982). 1,1-DCE was administered by gavage dissolved in corn oil (5 ml/kg in rats and 10 ml/ kg in mice) at doses of 1 and 5 mg/kg for rats and 2 and 10 mg/kg for mice. Small increases were seen in pheochromocytoma and interstitial-cell tumors of the testes in male rats and leukemia and pituitary adenomas in female rats. These increases were found not to be statistically different from control by life table analysis. No dose-related increases in tumor incidence was observed in mice. Quast et al. (1983) exposed Sprague-Dawley rats to 50, 100 or 200 mg 1,1-DCE/Liter of drinking water for 2 years. No exposure-related neoplastic changes were observed, although there was evidence of hepatocellular swelling and midzonal fatty change.

Despite the rather marginal carcinogenic effects of 1,1-DCE in animals there is ample evidence of its genotoxicity in a variety of test systems. Greim et al. (1975) were able to demonstrate mutagenic activity of 1,1-DCE in E. coli strain K12 in the presence of mouse liver microsomes. Similarly, Bartsch et al. (1975), Simmon et al. (1977), Cerna & Kypenova

(1977), Jones & Hathway (1978) and Oesch et al. (1983) demonstrated that 1,1-DCE were positive in a variety of S. typhimurium strains and that this activity was generally dependent upon metabolic activation.

The other two isomers, cis-1,2-DCE and trans-1,2-DCE, have been generally found negative under the same circumstances (Greim et al., 1975; Simmon et al., 1977). However, Cerna and Kypenova (1977) indicated in an abstract that cis-1,2 DCE produced positive results in a host-mediated assay using S. typhimurium strains TA1950, TA1951, and TA1952. These same authors indicated chromosomal damage in the bone marrow of female ICR Swiss mice with cis-1,2-DCE when administered at 1/2 the LD50 for 5 consecutive days. However, this data has only been reported in abstract form and is difficult to evaluate.

Although there appears to be adequate data to demonstrate the genotoxicity of 1,1-DCE in bacterial testor strains, the evidence for its carcinogenicity must be considered weak. Reports of positive findings of carcinogenic activity with 1,1-DCE have all been of a preliminary nature. Both studies involving lifetime observation (NTP, 1982; Quast et al., 1983) and oral exposures to 1,1-DCE were negative. Consequently, the evidence for the carcinogenicity of all isomers of dichloroethylene must be considered as minimal. However, it should be pointed out that the dosage levels used in carcinogenesis bioassays were quite low compared to similar studies conducted with related chemicals. These doses approximated maximally tolerated doses. In view of 1,1-DCE's genotoxicity, one is forced to consider the possibility that its toxicity prevents adequate testing of its carcinogenicity in experiments with the usually employed group sizes of 50 animals/sex oar less.

TRICHLOROETHYLENE:

Trichloroethylene (TCE) was first shown to be carcinogenic in a study sponsored by the National Cancer Institute (NCI, 1976). In this study, male B6C3F1 mice were exposed to time-weighted average doses of 1169 and 2339 mg/kg body weight, whereas, females received 869 and 1739 mg/kg of TCE dissolved in corn oil by gavage for a period of 78 weeks. In the same study both male and female Osborne-Mendel rats were administered doses of 549 and 1097 mg/kg TCE by the same means and for the same duration of exposure. Dose-related increases in the incidences of hepatocellular carcinomas were observed in both male and female mice, although the response was much more marked in the males. There were no statistically significant increases in tumor yield in either male or female Osborne-Mendel rats.

Inhalation studies conducted by Henschler et al. (1980) at concentrations of TCE in air of 100 and 500 ppm for 6 h/day, 5 days/week for a period of 18 months demonstrated an increased incidence of malignant lymphoma in female NMRI mice but was without apparent effect in male mice, Wistar rats or Syrian hamsters. More recently, Fukuda et al. (1983) were able to show a small increase in the incidence of pulmonary adenocarcinomas in female ICR Swiss mice exposed to 150 and 450 ppm TCE (16 and 15%, respectively, versus a control incidence of 2%) in air 7 h per day, 5 days a week for 104 weeks. No evidence of carcinogenic activity was observed in female Sprague-Dawley rats subjected to the same treatment. Consequently, evidence that TCE is carcinogenic is limited to mice, although three different tumors have been identified in the each of the mouse strains that have been studied to date.

As a result of the initial observation that TCE was carcinogenic in mice and because it comes from the same chemical class as vinyl chloride (chloroethylene), an established human carcinogen, there have been many studies of the compound's genotoxic properties. Simmon et al. (1977) reported that TCE increased the reversion rate of S. typhimurium strain TA100 only in the presence of a 9000 X g supernatant of homogenates made of mouse or rat liver. The experiments in this study were conducted in a desiccator and avoided the volatilization of TCE that is encountered in the standard plate assay of the Ames test when such precautions are not taken. Cerna and Kypenova (1977) were unable to demonstrate mutagenic activity of TCE in the spot test utilizing a variety of strains of S. typhimurium in the absence of a metabolic activation system, but were able to demonstrate positive activity in host-mediated assays in female ICR mice using strains TA1950, TA1951 and TA1952. Greim et al. (1975) demonstrated that TCE was capable of more than doubling the spontaneous mutation rate at the arg+ locus of E. coli strain K12 after incubation with a liver microsomal preparation taken from mice that had been pretreated with phenobarbital. No activity was attributable to TCE in the absence of metabolic activation. Henschler et al. (1977) failed to confirm the mutagenic activity of TCE in S. typhimurium strain TA100 both in the presence and absence of a metabolic activation system derived from Aroclor 1254 pretreated rats.

In addition to studies in bacterial systems, TCE has been shown to induce mutations in one yeast system by two different research groups (Shahin & von Borstel, 1977; Bronzetti et al., 1978) either in the presence of a metabolic activation system or in host-mediated assays, but negative

under similar circumstances in another (Rossi et al., 1983). Price et al. (1978) were able to demonstrate that TCE was capable of transforming Fischer rat embryo cells. Transformed cells were capable of growth in semisolid agar and produced undifferentiated fibrosacromas when inoculated into newborn Fischer rats. Henschler et al., (1977) suggested that the mutagenic activity and the carcinogenic activity of TCE might be accounted for by the presence of epichlorohydrin and 1,2-epoxibutane. However, these chemicals produced a mutagenic effect in the absence of a metabolic activation system, an observation that was not observed in prior positive tests of TCE.

It has been clearly shown that TCE is metabolized to a form capable of covalently interacting with tissue proteins at a level comparable to that observed with vinyl chloride (Van Duuren & Banerjee, 1976; Bolt & Filser, 1977; Allemand et al. 1978). It has not been possible to demonstrate a significant level of interaction with DNA in vivo (Bergman, 1983). Under in vitro conditions and in the presence of a metabolic activation system, it is possible to demonstrate binding of a TCE metabolite to calf thymus DNA (Bergman, 1983).

The evidence that TCE is carcinogenic was considered to be limited by IARC (1979d). At that time the evidence that TCE has genotoxic properties was obscured by reports of impurities present in the technical grade solvent (Henschler et al., 1977). However, the mutagenic activity associated with impurities have always shown activity in the absence of metabolic activation, whereas samples of TCE have been routinely negative in the absence of metabolic activation. As indicated above, positive results

have been specifically observed under circumstances where metabolic activation was possible in E. coli, S. typhimurium, and Saccharomyces cerevisiae. Demonstration of transformation in Fischer rat embryo cells supports the notion that TCE is able to act as a carcinogen. Negative results using bacterial and yeast systems in vitro appear to involve failure to consider the volatility of TCE or employed lower concentrations than utilized in positive studies. The reasons for the discrepancy in results using two yeast strains in host-mediated assays is not as clear. A lack of clear evidence of TCE interaction with DNA in vivo, however, presents a problem in invoking a genotoxic event as being responsible for the carcinogenic response. The difficulty in demonstrating such an interaction is in sharp contrast to that observed with vinyl chloride, a closely related chemical (Green and Hathway, 1978). On the other hand, it is difficult to rule out a minor interaction based upon in vitro evidence that such reactions are possible (Bergman, 1983).

Based largely on the newer observations of pulmonary adenocarcinomas (Fukuda et al., 1983) and malignant lymphoma (Henschler et al., 1980) in addition to hepatocellular carcinomas previously reported (NCI, 1976) in mice it must be concluded that there is adequate evidence that TCE is a carcinogen in mice. It should be pointed out, however, that these tumors have an appreciable background incidence in the strains of mice utilized. Along these lines, the reader is referred to the section on chloroform which suggests that the ability to extrapolate the liver tumors observed in mice subsequent to TCE administered in corn oil to other species may be limited.

TETRACHLOROETHYLENE:

To date the evidence that tetrachloroethylene (perchloroethylene, PCE) is carcinogenic is limited to a study conducted by the National Cancer Institute (1977). The results of this study indicated that time weighted average doses of 536 and 1072 mg/kg to male and 386 and 772 mg/kg given to female B6C3F1 mice by gavage using a corn oil vehicle for 78 weeks resulted in the development of hepatocellular carcinomas. No evidence of increased tumor incidence was observed in Osborne-Mendel rats given time weighted average doses of 471 and 941 mg/ kg in males and 474 and 949 mg/kg in females administered under the similar conditions in the same study. Rampy et al. (1978) also failed to observe increased tumor incidences in Sprague-Dawley rats exposed to 600 ppm TCE in air for a period of 12 months.

Evidence that PCE has genotoxic properties is also limited. PCE failed to increase the mutation frequency of E. coli K12 strain (Greim et al. 1975) in the presence or absence of mouse liver microsomes. It was reported (in abstract form) to produce an increase in the reversion rate of S. typhimurium strain TA100 in the absence of metabolic activation in the spot test, but was inactive in all other strains employed (Cerna & Kypenova, 1977). On the other hand, these same authors indicated that PCE was positive in a host-mediated assay utilizing S. typhimurium strains TA1950, TA1951 and TA1952 in female ICR mice at the LD50 and 1/2 of the LD50 in the same abstract. There was no evidence of dose-response. Bronzetti et al. (1983) were unable to demonstrate an effect of PCE using Saccharomyces cerevisiae strain D7 in a suspension assay in the presence or absence of mouse liver S-9 fraction or in a host-mediated assay. It

is notable that these same authors had previously demonstrated positive results with TCE under both of these conditions (Bronzetti et al. 1978).

The IARC (1979e) evaluated the evidence that PCE was carcinogenic and found only limited evidence that it was carcinogenic in mice. There is no substantive basis on which to modify that opinion at the present time. The reader's attention is, however, called to the discussion in the chloroform section with respect to the induction of hepatocellular carcinomas with hepatoxic agents administered with corn oil as a vehicle that bears on the question of whether such information can be extrapolated to other species.

BY-PRODUCTS OF TREATMENT PROCESSES

The trihalomethanes were the first chemicals that were recognized to be by-products of the chlorination of drinking water (Bellar et al., 1974; Rook, 1974). The most common forms of trihalomethanes found in drinking water include trichloromethanes (chloroform), dichlorobromomethane, dibromochloromethane, and tribromomethane (bromoform). Occasionally iodinated derivaties are also observed. Of this class of chemicals, the toxicologic effects of chloroform have been best characterized and have been previously reviewed by IARC (1979). A more recent, comprehensive review of its carcinogenic, mutagenic, and tetratogenic effects has been published by Davidson et al., (1982). The present section will primarily concern itself with data that has become available since those earlier reviews.

More recently, it has become quite apparent that the trihalomethanes are only one class of by-products produced in the chlorination of drinking water. Many of these by-products have been shown to be mutagenic in bacterial systems, but of these only the haloacetonitriles and chlorinated phenols have been shown carcinogenic in experimental animals. Thus, only these two additional classes will be covered in the present review. However, it should be remembered that chlorination is the only practice extensively studied. Many by-products remain to be identified with chlorine as well as other alternative modes of disinfection and even those which have been identified have been poorly characterized toxicologically.

In addition to by-products of drinking water disinfection, other treatment processes have the potential for the introduction of carcinogens into drinking water. In this light the carcinogenicity of two chemicals, epichlorohydrin and acrylamide, are discussed for illustrative purposes.

TRIHALOMETHANES:

Chloroform

The principal evidence that chloroform possesses carcinogenic activity arises from a study sponsored by the National Cancer Institute (NCI, 1976) where B6C3F1 mice of both sexes were shown to develop hepatocellular carcinomas and male Osborne-Mendel rats developed renal tumors following chronic exposure to chloroform (Table 3). These studies were conducted utilizing stomach gavage in a corn oil vehicle. Reanalysis of the histological slides from this study by Reuber (1979) revealed that the chloroform treated mice had a greater incidence of malignant lymphoma while the

rats also had developed thyroid tumors, cholangiofibromas and cholangiocarcinomas (females only).

For a variety of reasons, it was felt by some that the results of the NCI study were inadequate for purposes of estimating the cancer risk that chloroform in drinking water posed for humans. Central to this argument was the early observation by Eschenbrenner and Miller (1945) that hepatoma development in strain A mice following chloroform administration was confined to doses that produced frank liver necrosis. In order to address this and other issues, a larger study was co-sponsored by the U. S. Environmental Protection Agency and the National Cancer Institute (hereafter, the EPA-NCI study). This study differed from the previous NCI study in that chloroform was administered in the drinking water and the treatments were extended to lower doses with substantially expanded group sizes. This design was to avoid problems that may have been associated with gavage dosing and the corn oil vehicle. The expanded group size at low doses would provide a better basis for extrapolation of the results to estimate human risks. In addition, serum and tissue biochemical measurements were made in animals on parallel treatments. However, because of the aversive reaction to chloroform in drinking water it was necessary to include control groups that were restricted in the water consumption to the amount consumed by the high dose groups for each species. To avoid multiplying the costs of this large study, only female B6C3F1 mice and male Osborne-Mendel rats were included in the experiment.

The EPA-NCI study substantially supported the previous results obtained in the male Osborne-Mendel rat. In particular, there was a dose-related increase in renal tubular adenomas and adenocarcinomas. On

the other hand, results obtained with the female B6C3F1 mice were substantially different than observed in the original NCI study. The primary difference was the complete absence of any dose-related increases in the incidence of hepatocellular carcinomas. This result is particularly significant, since the daily doses of chloroform in the two high exposure groups bracket the low dose which gave rise to an 80% incidence of hepatocellular carcinomas in the earlier NCI study. It should be pointed out that exposures were continued through 104 weeks in the EPA-NCI study compared to a duration of only 78 weeks for the prior NCI study.

Table 3 summarizes the available data dealing with the carcinogenic effects of chloroform in experimental animals. It is notable that those studies that were unable to demonstrate any carcinogenic activity with chloroform treatment uniformly involved dosage levels lower than those used in either the NCI or the EPA-NCI studies. The only positive study not previously mentioned was the finding of increased kidney tumors in ICI mice when chloroform was administered in a toothpaste base at a dose of 60 mg/kg per day (Roe et al. 1979). Renal tumors were not produced in the C57BL, CBA, and CF/1 strains at the same dose in the same study. It is notable that liver tumors were not observed in any of these mouse strains.

Because of the high doses that have been required to produce tumors with chloroform treatment, it has been argued that its carcinogenicity was secondary to tissue necrosis followed by regenerative hyperplasia. Reitz et al. (1982) were able to show that doses of 60 and 240 mg of chloroform /kg do produce substantial increases of tritiated thymidine incorporation into DNA of both the liver and kidney of the B6C3F1 mouse.

These data indicate a regenerative response and are consistent with this hypothesis. Moore et al. (1982) found that doses approximating 60 mg/kg in corn oil resulted damage to the kidney on evidence of tubular regeneration as measured by tritiated thymidine uptake. Similar doses in a toothpaste base was without effect. At 240 mg/kg similar effects were seen with both vehicles. Such responses were, however, not strongly apparent in the liver or kidney of Osborne-Mendel rats.

In the EPA-NCI study, there was little evidence of overt hepatic or renal pathology in the B6C3F1 mice treated with chloroform. However, there was some evidence that the liver was unable to adequately handle triglyceride. There was a dose-related increase in the liver fat content apparent in chloroform-treated mice that was observed only at much higher doses in the rat. In the rat these changes in liver fat were preceded by substantial a depression of serum triglycerides that became particularly apparent in the aging rats. Data on serum triglycerides of the mice under these conditions are not presently available. At this time one can only speculate that chloroform's compromize of the liver's ability to handle the additional lipid load resulting from the use of corn oil may have contributed to the development of hepatocellular carcinomas in the first NCI study.

The argument that chloroform only induced tumors secondary to overt tissue necrosis is indirectly supported by the difficulty involved in demonstrating evidence of genotoxic properties (IARC, 1979f). More recent data (Table 4) has confirmed the lack of direct association of chloroform or a metabolite with DNA in the mouse or rat liver or kidney

(Diaz-Gomez and Castro, 1980; Reitz, et al. 1982; Pereira et al. 1982). However, it has been shown that chloroform possesses the capability of inducing chromosome breakage and increased sister chromatid exchange frequencies in human lymphocytes in vitro and increased sister chromatid exchange in the bone marrow of mice treated with chloroform in vivo (Morimoto and Koizumi, 1983). In addition, chloroform has been shown to enhance the transformation of Syrian Hamster Embryo (SHE) cells by S7 adenovirus (Hatch et al., 1983). It is notable that the concentrations of chloroform tested in these positive experiments tend to be higher than those utilized in similar experiments that had negative results (Kirkland et al., 1981). The interpretative difficulties that are raised by the inability to produce strong evidence of a genotoxic effect of chloroform is further complicated by the fact that attempts to demonstrate an alternative mechanism for chloroform-induced cancer have produced equivocal data. For example, Pereira et al. (1982) failed to clearly demonstrate either tumor initiating or tumor promoting activity in the rat liver. The available data relevant to the potential genotoxicity of chloroform is summarized in Table 4.

In estimating the carcinogenic risk that chloroform in drinking water presents to man all the above data must be considered. At present it is clear that chloroform is capable of producing renal tumors in at least one strain of mice and rats. Under circumstances involving gavage with vehicles with a high lipid content, chloroform can also clearly induce liver tumors. These data are sufficient to conclude that chloroform is probably carcinogenic in humans. However, in view of the apparent dependence of the carcinogenic effects of chloroform in the mouse liver

on the mode of administration, it is suggested that chloroform's tumorogenic activity in the rat may be more appropriate for estimating carcinogenic risk in humans.

HALOACETONITRILES:

To date the haloacetonitriles that have been identified in chlorinated drinking water have primarily involved the dihalogenated chlorine and bromine derivatives (Trehy & Bieber, 1981; Oliver, 1983). The occurence of the haloacetonitriles in water at the tap is at least partially dependent upon the pH of the finished drinking water, since all members of the class normally found in drinking water are alkaline-labile (Bieber & Trehy, 1983).

Investigations into the carcinogenicity of these chemicals has been confined to the three members of the chlorinated series, bromochloroacetonitrile and dibromoacetonitrile. The first experimental evidence of the potential for these derivatives to act as chemical carcinogens was the observation that dichloroacetonitrile (DCAN) was mutagenic in Salmonella typhimurium (Simmon et al., 1977). More recently, Bull et al. (1984) have confirmed the mutagenic properties of DCAN in Salmonella and shown that bromochloroacetonitrile (BCAN) is mutagenic under similar circumstances. Chloroacetonitrile (CAN), trichloroacetonitrile (TCAN) and dibromoacetonitrile (DBAN) were found to be inactive in the presence or absence of a 9000 X g supernatant fraction (S-9) of an liver homogenate taken from rats previously treated with Arochlor 1254. On the other hand, all five haloacetonitriles tested significantly increased the frequency of sister chromatid exchange (SCE) in Chinese hamster ovary

cells, in vitro, in the absence of rat liver S-9 fraction. The potencies of the haloacetonitriles to induce SCE (induced SCE/mMolar concentration): CAN, 50; DCAN, 103; TCAN, 230; BCAN, 396; and DBAN, 469. None of these same five haloacetonitriles could be shown to possess mutagenic activity in the mouse micronucleus assay in vivo (5 male and 5 female CD-1 mice in each group) at doses of 0, 12.5, 25 and 50 mg/kg bw for 5 consecutive days in this same study.

To date, direct evidence of the carcinogenicity of these chemicals is limited to experiments where the five above-named compounds were tested as tumor initiators in the mouse skin. Experiments employing the oral route of administration (a total dose of 150 and 300 mg/kg bw split between 6 individual doses) followed by a 20 week promotion schedule with 12-0-tetradecanyl-phorbol-13-acetate (TPA) failed to demonstrate a significantly elevated incidence of either benign or malignant skin tumors within a 1 year observation period (Bull et al., 1984). On the other hand, higher doses applied topically (1200, 2400 and 4800 mg/kg, also split between 6 applications), yielded significantly higher tumor incidences with CAN, BCAN and DBAN. DCAN and TCAN produced small increases in cumulative tumor yields, but the changes were not significantly different from controls.

These data indicate that certain members of the haloacetonitrile class do possess carcinogenic properties. To this point, in time, this evidence has been confined to the topical route of administration and coupled to the subsequent application of a potent tumor promoting agent, TPA. There is no evidence available concerning the ability of these

compounds to induce cancer by a systemic route of administration although there are experiments currently being conducted in strain A/J mice (Bull, et al., 1984a). Because of their relatively common occurrence as a result of the chlorination of drinking water it is critical that studies examining this issue be initiated.

CHLORINATED PHENOLS:

Chlorination of drinking water often gives rise to small quantities of chlorinated phenol derivatives. The principle products are 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. There is substantive evidence of carcinogenic properties for only 2,4,6-trichlorophenol. The National Cancer Institute (NCI, 1979) conducted a feeding study of the effects of 5,000 and 10,000 ppm of 2,4,6-trichlorophenol in the diet of F344 rats and 5,214 and 10428 ppm in the diet of B6C3F1 mice. The incidence of lymphomas or leukemias was increased in male rats in a dose-related manner. Hepatocellular carcinomas were increased in both male and female mice and the incidences were dose-dependent. As a consequence of this study, it is reasonable to conclude that 2,4,6-trichlorophenol may well represent a carcinogenic hazard to man.

OTHER TREATMENT CHEMICALS:

A wide variety of chemicals are necessary in the production of a safe and wholesome drinking water. For the most part these chemicals are generally regarded as safe and pose no substantive hazard to the health of the consuming public. However, if the synthesis of some products is improperly controlled they can contain contaminants that are

of potential concern. An example of such products is the group of polymeric chemicals that are commonly used clarifying water, the coagulant aids.

The coagulant aids are high molecular weight polymers that are essentially non-toxic and are removed almost quantitatively from the finished water by precipitation and filtration. However, the monomeric components used in the synthesis of these polymers are often reactive chemicals that are highly toxic and if sufficiently stable in water could be expected to be in the finished drinking water. One example of such a monomer is epichlorohydrin.

Evidence for carcinogenicity of epichlorohydrin was considered to be sufficient by IARC (1982b) based on its ability to induce forestomach tumors in male rats when administered orally (Konishi et al., 1980) and inducing papillomas and carcinomas of the nasal cavity following inhalation exposure (Laskin et al., 1980). In addition, it has been shown capable of inducing DNA damage (it is a directly active alkylating agent) in a variety of prokaryotic and eukaryotic systems, it induces mutation and chromosomal anomalies in both plant and mammalian systems (IARC, 1982).

Acrylamide is a second chemical that has been commonly used in the synthesis of a number of coagulant aids. It also is used in a new in situ polymerization process that is injected into the ground as a grouting technique. This latter use could serve as a source of ground water contamination with acrylamide. Acrylamide has been recently shown capable of initiating papillomas and carcinomas in the skin of mice whether administered by the oral, intraperitoneal or topical routes of adminstra-

tion (Bull et al., 1984b). It was also shown to be capable of increasing the yield of lung adenomas in strain A/J mice in the same study. A large study in rats has been recently completed by a consortium of manufacturers of coagulant aids that has been reported significant increases in tumors in a variety of organs. This work has yet to be reported in the open literature.

Although acrylamide is inactive in the standard plate assay of the Ames' test (Bull et al., 1984b), it does increase the frequency of sister chromatid exchange in Chinese hamster ovary cells, in vitro (Bull et al., 1983). Shiraishi and Yamamoto (1978) and Shiraishi (1978) reported that acrylamide produces aneuploid and polyploid cells in both bone marrow cells and spermatogonia of mice treated with acrylamide by oral or intraperitoneal administration. Bull et al. (1983) observed dose-related increases in spermhead abnormalities in mice with acrylamide. More recently, Vanhorick and Moens (1983) found that acrylamide was a weak inducer of SV40 DNA amplification in SV40-transformed Chinese hamster cells as measured by in situ hybridization techniques. However, acrylamide synergistically enhanced the induction of SV40 DNA synthesis by a number of other carcinogens.

From these data, it appears that acrylamide is carcinogenic in both rats and mice. It would, therefore, be prudent to consider it as a potential carcinogenic hazard to man.

DISTRIBUTION SYSTEM

Contribution of toxic chemicals from the distribution system tc finished drinking water can be considerable under certain circumstances. It is beyond the scope of this paper to deal comprehensively with this subject. However, it should be pointed out that certain products used in drinking water distribution are made of monomeric components that are recognized carcinogens. The most obvious example is polyvinyl chloride pipe which is a polymer of the well established human carcinogen vinyl chloride (IARC, 1979). Modern manufacturing techniques have reduced the monomer content of these products to negligible concentrations, but users of such products should carefully avoid substandard materials.

Another product that was widely used in lining of certain water mains, storage tanks and pipes are coal tar and asphaltic-based paints. The high polyaromatic hydrocarbon content of coal tar paints is partially, but not completely responsible for the high carcinogenic activity of these products in the mouse skins (Robinson et al., 1984). Although still containing carcinogenic components, the asphaltic based paints used in contact with potable water are approximately 3 orders of magnitude less potent than their coal tar analogs. Because of the extremely low water solubility of the the polyaromatic hydrocarbons that are partially responsible for the carcinogenic activity of coal tar these chemicals would not be expected to be rapidly leached from coatings. However, the extent to which these coatings provide a relatively constant reservoir for the leaching of carcinogen from the surface of the distribution system has not been well studied. However, some information exists to

suggest that as these coatings age they can begin to shed fine particulates containing high concentrations of polyaromatic hydrocarbons into the finished drinking water.

DISCUSSION

It is clear from the previous discussion that chemical carcinogens find their way into drinking water through a variety of means. Simplistic views on how these chemicals get into finished drinking water are to be avoided. Obviously, industrial contamination is only one and in many cases a minor source of chemicals in drinking water. Carcinogenic properties of the chlorination by-products 2,4,6-trichlorophenol and the haloacetonitriles indicate that the view that trihalomethane concentrations represent the carcinogenic hazard associated with by-products of disinfection must be abandoned. Finally there is a clear need to more carefully scrutinize products that are utilized in the treatment and distribution of drinking water since these products can be a major source of harmful chemicals presented at the tap for human consumption.

While it is not possible to be certain of the absolute risk to humans exposed to traces of these substances found in drinking water, a prudent policy is to minimize both the frequency and concentrations at which they occur. Prudence also dictates that chemicals that are clearly identified as carcinogens in lower mammals must for all intents and purposes be considered as carcinogenic hazards to man. The basis for this attitude is that all chemicals that have been identified as carcinogens except one (arsenic) have been shown carcinogenic in lower mammals. Since man cannot be exposed to chemical carcinogens in a controlled

manner, direct documentation of cancer causing agents in man is generally limited to epidemiological information. Due to the difficulties associated with separating out low level risks attributable to individual chemicals in the general environment the ability to identify chemical carcinogens by epidemiological investigations is limited. Since the dose of a potential carcinogen to which an experimental animal can be exposed is limited only by its acute and/or chronic toxicity, the ability to identify carcinogenic substances is much more straightforward. As a consequence the data available on chemicals that can produce cancer in animals, although in itself far from complete, is much greater than that available directly documenting carcinogenic effects in man.

Despite the need to accept the general notion that carcinogenicity in experimental animals signals cause for concern about human exposures to the same chemical, it must be recognized that the considerable uncertainty underlies the extrapolation of that information to man. These uncertainties are both qualitative and quantitative and have been discussed in detail elsewhere (IARC, 1982c). The present paper was not intended to deal with these issues in general, but to focus primarily on questions that specifically involve selected contaminants of drinking water.

If a chemical induces malignant tumors in more than one species, particularly if it produces tumors that are rare in the particular strain or species its carcinogenic properties are widely accepted (IARC, 1982c). On the other hand, a chemical that increases the incidence of a tumor that has a high spontaneous incidence in the test animal employed are felt to provide only limited evidence of carcinogenicity. Certain of

the low molecular weight chlorinated hydrocarbons fall into this category. Other chemicals from the same class, such as vinyl chloride, cause little controversy because they produce malignant tumors in multiple sites and different species. It should be recognized that this view does not suggest that increased incidence of spontaneous tumors is of less concern than induction of rare tumors. Rather it is a statement related to the wide variety of factors that can modify the yield of a spontaneous tumor, whereas induction of a rare tumor clearly involves the properties of a complete carcinogen. In modern times, complete carcinogenicity has come to include genotoxic activity, although there are certainly other biological properties that are essential to the expression of chemically-induced cancer (Weisburger and Williams, 1983).

It is ironic that most of the chemicals that occur frequently in drinking water fall into a class of carcinogens whose carcinogenic effects are controversial. In general, the controversy involves those chemicals which induce liver tumors, in particular those whose activity is limited to the induction of liver tumors in mice. Part of this concern is illustrated by the fact that the yield of hepatocellular carcinomas in B6C3F1 mice can be increased substantially by simple performance of a partial hepatectomy (Newberne et al., 19---). The chemicals most directly involved in this controversy are trichloroethylene and tetrachloroethylene, but also involve considerations of the carcinogenicity of carbon tetrachloride and chloroform.

Carbon tetrachloride should in many ways be considered the prototype for this group, although its carcinogenicity seems somewhat more generally accepted than the other three compounds. As pointed out above, carbon

tetrachloride is capable of producing liver tumors in a variety of species. However, despite many attempts (many of which have not been published), it has been virtually impossible to demonstrate any genotoxic activity with carbon tetrachloride (IARC, 1979b; USEPA, 1984).

In general, studies that have demonstrated carbon tetrachloride induced liver cancer has involved very high doses, from 1000 to more than 2000 mg/kg administered in an vegetable oil vehicle 2 to 5 times weekly for extended periods of time. In one case where it was stated that the carcinogenic responses to carbon tetrachloride do not correlate with the degree of hepatic injury, the strains of rat not responding with a carcinogenic response were those which died early because they were most sensitive to the hepatoxic effects of carbon tetrachloride (Reuber and Glover, 1970). Since, only one dose level was used for all strains in this study, there was no real opportunity to determine the relative sensitivity of these strains to carbon tetrachloride-induced hepatocellular carcinomas.

Eschenbrenner and Miller (1946) were able to dissociate the induction of hepatomas from overt necrotic change by manipulating the dosage schedule for carbon tetrachloride in mice. On the other hand, these investigators were not able to rule out a low level of cell damage and a resulting increased level of cell replacement; although they did examine the effects necrotizing doses of carbon tetrachloride on the numbers of mitotic figures in the liver. They did find evidence of cirrhosis at doses not involving obvious necrosis but resulting in hepatoma induction. Recent data indicates that both mice and rats subjected to repeated doses

of carbon tetrachloride as low as 10 mg/kg per day results in substantial evidence of liver injury when administered over a 90 day period. This dose is approximately one-half the lowest dose that Eschenbrenner and Miller found capable of inducing hepatomas over a somewhat greater time interval. It is notable that Eschenbrenner and Miller's data do show that administration of higher doses of corn oil with carbon tetrachloride enhances liver necrosis produced by carbon tetrachloride.

The experimental results with trichloroethylene in many ways contrast sharply with that obtained with carbon tetrachloride. Trichloroethylene has been shown to only induce hepatocellular carcinomas in mice (NCI, 1976). Although of a rather low potency, the genotoxic properties of trichloroethylene have been demonstrated by a number of investigators. In this respect, evidence of its carcinogenic activity would have to be considered stronger than that of carbon tetrachloride. However, the fact remains that trichloroethylene has only been shown capable of inducing liver tumors in mice.

The paradox between the lack of clear evidence that CCl_4 is genotoxic and its demonstrated carcinogenicity and the much better evidence that TCE is genotoxic but with weak evidence of carcinogenicity may be partially accounted for by results of the recent EPA-NCI study of chloroform-induced cancer. In the first NCI study, chloroform was administered by gavage in corn oil and produced a high incidence of hepatocellular carcinomas in both male and female B6C3F1 mice. In the second EPA-NCI study, comparable doses of chloroform were administered via the drinking water and failed to produce an increased incidence of hepatocellular carcinomas in the same strain of mice. The hypothesis that gavage with corn oil results in

higher effective doses of these chemical by increasing absorption has been clearly eliminated. Withey et al., (1983) showed that peak blood concentrations were much lower following intragastric administration in corn oil vs. water as the vehicle for chloroform, TCE and other chlorinated hydrocarbons. Perhaps a better explanation is to be found in the fact that incorporation of corn oil into the diet has been shown to increase the yield of liver tumors in aflatoxin B_1-initiated rats (Newberne et al., 1979). These data suggest the distinct possibility that tumors observed with chemicals found in drinking water may well have involved an interaction with the vehicle.

Examination of the livers of mice receiving chloroform in drinking water demonstrated a dose-related increase in liver fat content that extend to levels much lower than those utilized for the original NCI carcinogenesis study. Similar changes were not observed in the livers of Osborne-Mendel rats until much higher doses of chloroform were utilized. These results suggest that corn oil was in some way involved in the of the liver pathology that develops in mice treated with chloroform. Perhaps the relatively minor damage implied by the small accumulation of liver fat in mice treated with chloroform is substantially enhanced by the high levels of corn oil used in the gavage protocol for non-water soluble chemicals in the National Toxicology Program's bioassay procedures. If so, this would imply that tumors induced by other hepatoxic agents using this protocol may depend heavily on the corn oil vehicle. Such an interpretation would suggest that results that have been obtained

with chemicals such as trichloroethylene and tetrachloroethylene may not be extrapolated to other species without appropriate qualification. In fact, these data also call into similar question the carcinogenicity of carbon tetrachloride since it has only been shown capable of producing liver tumors when administered in a vegetable oil vehicle. As was pointed out above, doses of carbon tetrachloride lower than those shown to induce liver tumors produce definite liver pathology in both rats and mice.

In the case of tetrachloroethylene, increases in hepatic DNA synthesis is observed in B6C3F1 mice but not in rats following doses of 1000 mg/kg per day for 11 days (Schumann, et al., 1980). Similarly, Reitz et al. (1982) found substantial increases in DNA synthesis of the liver and kidney of B6C3F1 mice treated with single doses of 60 and 240 mg/kg of chloroform and much smaller increases in the liver and kidney of male Osborne-Mendel rats. These data indicate that the toxic effects of these chemicals in the mouse liver results in substantial tissue regeneration at dose levels utilized in the carcinogenesis bioassays of these chemicals. Consequently, the possibility exists that these chemicals are capable of increasing liver tumor development from spontaneously initiated cells in the same way that carbon tetrachloride has been shown to increase hepatomas in the liver of diethylnitrosamines initiated mice (Pound and McGuire, 1978).

In summary, it must be said that evidence that a chemical induces cancer in experimental animals suggest that there is a potential risk to man regardless of the mechanism involved. Although it is certainly possible that a chemical that produces cancer in experimental animals

would not do so in man, it is difficult to develop a prudent course of action on this assumption because the evidence which presently exists would suggest that the reverse is the general case. If tumorogenesis is dependent upon tissue damage, it should be remembered that most of the agents of concern have been shown capable of inducing liver damage in humans. Consequently, it should be recognized that the argument presented regarding relationship of tissue damage to tumorigenicity is probably more of a quantitative consideration in the judgement of risks in man and is less of a question of across-species extrapolation than discussions of the issue would ordinarily imply. Consequently, the appearance of all the above chemicals in drinking water is cause for concern and to be avoided wherever possible and practical.

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Table 1. Evidence for Carcinogenicity of Selected Chemicals that Contaminate Sources of Drinking Water

Chemical	Species & Strain (Sex)	Highest Dose	Route	Vehicle	Result	Reference
Carbon Tetrachloride	C3H Mice (M&F)	0.04 ml 2 to 3 times weekly for 8-16 weeks	oral	Olive oil	Hepatoma	Edwards, et al., 1941
	Strain L	0.04 ml 46x over a 4 month period (no controls)	"	"	Hepatoma	Edwards, et al., 1941
"	Strain A Mice (M&F)	2.5g/kg to a total of 30 doses	Oral	Olive Oil	Hepatoma	Eschenbrenner & Miller, 1943
"	B6C3F1 Mice (M&F)	2500/mg/kg 5 X weekly for 78 wks	Oral	2-5% solution in corn oil	Hepatocellular carcinoma	NCI, 1976
"	C3H Mice	1.6 g/kg 3 X weekly for 10 wks	Oral	Corn Oil	Hepatoma	Kiplinger & Kensler, 1963
"	C3H Mice	0.04 ml 2 times weekly for 20-26 weeks	rectal	Olive Oil	Liver Tumors	Confer & Stenger, 1965
"	Osborne-Mendel Rat (M&F)	160 mg/kg 5 X weekly 78 weeks	Oral	Corn Oil	Negative	NCI, 1976
"	Syrian golden hamsters	12.5 ul/hamster weekly no controls	Oral	Corn Oil	liver cell carcinomas	Della Porta et.al, 1976
"	Japanese rat (M)	2g/kg bw 2 X weekly	s.c.	Corn Oil	Hepatocellular carcinoma	Reuber & Glover, 1970
"	Osborne-Mendel (M)	" " " "	s.c.	" " "	Hepatocellular carcinoma	Reuber & Glover, 1970
"	Wistar - (M)	" " " "	s.c.	" " "	Hepatocellular carcinoma	Reuber & Glover, 1970
"	Black rat (M)	" " " "			(Died early)	Reuber & Glover, 1970
"	Sprague-Dawley (M)	" " " "	"	" " "	"	Reuber & Glover, 1970

Table 1. (Continued) PAGE 2

Chemical	Species & Strain (Sex)	Highest Dose	Route	Vehicle	Result	Reference
1,1-Dichloroethylene	B6C3F1 Mice (M&F)	10 mg/kg 5 x weekly for 104 weeks	Oral	Corn Oil	Negative	NTP, (1982)
"	" Rats (M&F)	5 mg/kg 5 x weekly for 104 weeks	Oral	Corn Oil	Negative	NTP, (1982)
"	Swiss Mice (M&F)	25 ppm 4 h daily 4-5 times weekly (poor survival at higher doses) for 12 months	Inh.	Corn Oil	Renal Adeno-carcinomas	Maltoni, 1977
"	Sprague-Dawley Rats (M&F)	150 ppm 4 h daily 4-5 times weekly for 12 months	Inh.	Air	Mammary and total tumors	Maltoni, 1977
"	" "	20 mg/kg 4-5 times weekly for 52 weeks	Oral	Olive Oil	Negative	Maltoni, et al., 1977
"	Chinese Hamsters (M&F)	25 ppm 4 h daily 4-5 times a week for 12 months	Inh.	Air	Negative	Maltoni, 1977
"	CD-1 Mice (M&F)	55 ppm 6 h daily 5d/wk - 12 months	Inh.		Negative	Lee et al., 1978
"	CD Rats (M&F)	" " " " "	"		Negative	Lee et al., 1978
"	Sprague-Dawley Rats (M&F)	200 mg/L for 2 years	Drinking Water		Negative	Quast et al., 1983
Trichloroethylene	B6C3F1 Mice (M&F)	2339 mg/kg TWA for 78 weeks	Oral	Corn Oil	Hepatocellular carcinoma	NCI, (1976)
"	Osborne-Mendel (M&F)	1097 mg/kg TWA for 78 weeks	Oral	Corn Oil	Negative	NCI, (1976)

Table 1. (Continued) PAGE 3

Chemical	Species & Strain (Sex)	Highest Dose	Route	Vehicle	Result	Reference
Trichloroethylene	ICR Mice (F)	0, 150 & 450 ppm in air 7 h per day, 5 days a week	Inh.	Air	Pulmonary adenocarcinoma	Fukuda, et al., 1983
"	Sprague-Dawley rats (F)	0, 150 & 450 ppm in air 7 h per day, 5 days a week	Inh.	Air	Negative	" "
"	Mice (M&F)	500 ppm for 18 months	Inh.	Air	Malignant lymphoma	Henschler et al., 1980
"	Rats (M&F)	500 ppm for 18 months	Inh.	Air	Negative	" "
"	Hamsters (M&F)	500 ppm for 18 months	Inh.	Air	Negative	" "
Tetrachloroethylene	B6C3F1 Mice (M&F)	1072 mg/kg TWA for 78 weeks	Oral	Corn Oil	Hepatocellular carcinoma	NCI, (1977)
"	Osborne-Mendel (M&F)		Oral	Corn Oil	Negative	NCI, (1977)
"	Sprague-Dawley (M&F)	600 ppm in air for 12 months	Inh.	-	Negative	Rampy et al., 1978

Table 2. Evidence for Genotoxicity of Selected Contaminants of Sources of Drinking Water

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
1,1 - Dichloroethylene	E. Coli (K12)	gal + arg + nad + MTR	+/- mouse microsomes phenobarbital pre-treatment	2.5 mM	pos. pos. neg. neg.	Greim et al., 1975
"	S. Typhimurium	TA100	+/- mouse and Rat S-9 fraction in desiccators	5% in air for 12 hours	Pos. only with S-9	Simmon et. al., 1977
"		TA1950 TA1951 TA1952 TA1535 TA1538 TA100 TA98	Spot test - did not use S-9	0.05 ul/plate	pos. no DR	Cerna & Kypenova, 1977
"	"	"	Host mediated	LD_{50} and 1/2 LD_{50}	Pos. but no DR	" "
"	"	TA1535	+/- Mouse and Rat S-9 fraction induced and non-induced with Aroclor 1254 Human on Phenobarbital medication Human non-induced marmoset- " "	5% in air sealed	Pos. markedly increased with induction Human Pos. with Pheno-barb induction non-induced Human neg.	Jones and Hathway, 1978
"	"	TA100 TA1535	+/- Mouse and Rat tissue S-9 fractions - (QF-1 mouse BDVI rat - liver, kidney and lung tissues) Phenobarbital pretreated animals.	2 & 20% VDC in air	Species & tissue dependent pos. respon.	Bartsch et. al., 1975

Table 2. (Continued) PAGE 2

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
1,1 - Dichloroethylene	S. Typhimurium	TA1535 TA1537 TA98 TA100 TA92	+/- Liver and Kidney S-9 fraction from mice, rats and chinese hamsters Human autopsy tissue (liver)	375-22500 ppm	pos. pos. pos. pos. pos.	Oesch et al., (1983)
"	E. Coli WP2 uvrA					
cis -1,2-Dichloroethylene	E. Coli K12	gal + arg + nad + MTR	+/- mouse microsomes phenobarbital pretreatment	2.9 mM	neg. neg. neg. neg.	Greim et al., 1975
"	S. Typhimurium	TA1535 TA1537 TA1538 TA98 TA100	+/- Mouse and rat S-9 fraction	?	neg. neg. neg. neg. neg.	Simmon et al., 1977
"	"	TA1950 TA1951 TA1952	Host mediated		Pos. but which strain not clear	Cerna and Kypernova 1977
"	Mouse (ICR Female) bone marrow cytogenetics			5 doses-1/2 LD_{50}	Pos.	" "
trans-1,2-Dichloroethylene	E. Coli K12	gal + arg + nad + MTR	+/- mouse microsomes phenobarbital pre-treated	2.3 mM	Neg.	Greim et al., 1975
"	S. Typhimurium	TA1535 TA1537 TA1538 TA98 TA100	+/- Mouse and rat S-9 fraction	?	Neg.	Simmon et al., 1977

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
trans-1,2-Dichloroethylene	S. Typhimurium	TA1950 TA1951 TA1952 TA1535 TA1538 TA100 TA98	Spot Test	0.05 ml	Neg.	Cerna and Kypenova, 1977
"	"	TA1950 TA1951 TA1952	Host mediated	LD_{50} and 1/2 LD_{50}	Neg.	" "
Trichloroethylene	S. Typhimurium	TA100	+/- mouse and rat S-9 fraction in desiccators	1.5 ml in desiccator 2.5 ml cytotoxic	Pos. req. S-9	Simmon et al., 1977
"	"	TA1950 TA1951 TA1952 TA1535 TA1538 TA100 TA98	Spot test no S-9 used in vitro	0.05 ml/plate	Neg. neg. neg. pos. pos. neg. neg.	Cerna & Kypenova 1977
"	"	TA1950 TA1951 TA1952	Host mediated " "	LD_{50} & 1/2 LD_{50}	pos. pos. pos.	Cerna & Kypenova, 1977
"	E. Coli K12	gal + arg + nad + MTR (forward mutation)	+/- mouse microsomes phenobarbital pre-treated	3.3 mM in media	arg + pos. req. metabolic activation	Greim et al., 1975
"	Covalent binding in rat hepatic microsomes		In vitro		pos.	Van Duuren & Banerjee, 1976

Table 2. (Continued) PAGE 4

Chemical	Test System	Special Conditions	Highest Dose Tested	Result	Reference
Trichloroethylene	Covalent binding to tissue proteins in Wistar rats.	In vitro	1000 ppm for 5 hours. (approx. the same level of binding seen with vinyl chloride).	pos.	Bolt and Filser, 1977
"	Covalent binding to protein in rat liver microsomes	In vitro	Unlike vinyl chloride binding is not specific to SH containing protein.	pos.	"
"	S. Typhimurium TA100	+/- Aroclor 1254 induced rat liver S-9 fraction	100 ug/ml in top agar	neg. with and without S-9	Henschler et al., 1977
"	Saccharomyces cerevisiae XV185-14C	+/- mouse liver S-9 fraction	20 ul/ml	Pos. with S-9 only	Shahin & Von Borstel, 1977
"	Fischer Rat F1706 embryo cell system		1.1×10^4 μ M	Pos.	Price et al., 1977

Table 2. (Continued) PAGE 5

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
Trichloroethylene	Covalent binding to hepatic proteins in the S.D. Rat		In vivo and In vitro		Pos.	Allemand, et al., 1978
"	Saccharomyces cerevisiae D7		Suspension test, +/- mouse liver 10,000 x g supernatant	40 mM	Pos. point mut. and give conversion required S-10 to be active	Bronzetti, et al., 1978
"	"	D7	Host mediated	400 mg/kg	pos. ilv reversion + trp conversion	" " "
"	"	D4	" "	400 mg/kg	Pos. at ade and trp loc.	" " "
"	"	D4	Host mediated	3700 mg/kg (22 repeated doses of 150 mg/kg + 400 mg/kg day of sacrifice).	pos. trp and ade loci	" " " " " "

Table 2. (Continued) PAGE 6

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
Trichloroethylene	Schizosaccharomyces pombe	P1	+/- mouse and rat liver S-9 fraction pretreated phenobarbital or B-naphthoflavone	22 mM	neg. at ade loci 1,3,4, 5&9	Rossi, et al. 1983
"	Schizosaccharomyces pombe	P1	Host mediated	2 g/kg	neg.	Rossi, et al. 1983
"	DNA binding in in vitro & in vivo		Calf thymus, in vitro mouse, in vivo		pos. in vitro neg. in vivo	Bergman, 1983
Tetrachloroethylene	E. Coli K12	gal + arg + nad + MTR	+/- Mouse microsomes, phenobarbital pre-treated	0.9 mM in media	neg.	Greim et al. 1975
"	S. typhimurium	TA1950 TA1951 TA1952 TA1535 TA1538 TA100 TA98	Spot test-no S-9 used in vitro		neg. neg. neg. neg. neg. pos. neg.	Cerna & Kypenova, 1977
"	S. typhimurium	TA1950 TA1951 TA1952	Host mediated Female ICR mice	LD_{50} and 1/2 LD_{50}	Pos. no dose response evident	Cerna & Kypenova, 1977
"	Macromolecular binding in rat and liver tested in vivo			500 mg/kg (oral dose)	Binding to macro-molecules but no binding detected in purified DNA	Schumann et al. 1980

Table 2. (Continued) PAGE 7

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
Tetrachloroethylene	Saccharomyces cerevisiae	D7	Suspension assay +/- mouse liver S-9	85 mM	Neg.	Bronzetti et al. 1983
			Host mediated assay	11 g/kg (single dose) 2g/kg x 12 + 4 g/kg	Neg.	
					Neg.	

Table 3. Evidence for Carcinogenicity of Chloroform in Experimental Animals

Species and Strain	Vehicle	Highest Dose Tested	Route	Tumor	References
A Mice	Olive Oil	2350 mg/kg	oral	Liver	Eschenbrenner & Miller, 1945
C57BL Mice	Toothpaste	60 mg/kg/d	oral	none	Roe et al., 1979
CBA Mice	Toothpaste	60 mg/kg/d	oral	none	"
CF/1	Toothpaste	60 mg/kg/d	oral	none	"
ICI Mice	Toothpaste	60 mg/kg/d	oral	Kidney	"
Sprague-Dawley Rat	Toothpaste	60 mg/kg/d	oral	none	"
Beagle Dog	Toothpaste	20 mg/kg/d	oral	none	"
"	"	"	"	Total Neoplasms	Reuber, 1979
Osborne-Mendel Rat	Corn Oil	180 mg/kg/d	oral	Kidney	NCI, 1976
"	"	"	"	Thyroid	Reuber, 1979
"	"	"	"	Cholangiofibromas	" "
"	"	"	"	Cholangiocarcinomas	" "
B6C3F1 Mice	Corn Oil	477 mg/kg/d	oral	Liver	NCI, 1976
"	"	"	"	Lymphoma	Reuber, 1979
Osborne-Mendel Rat	Drinking Water	130 mg/kg/d	oral	Kidney	EPA-NCI Study.
B6C3F1 Mice	Drinking Water	400 mg/kg/d	oral	None	EPA-NCI Study.

Table 4. Evidence for Genotoxic Activity of Chloroform

Test System		Special Conditions	Highest Dose Tested	Result	Reference
S. Typimurium	TA1535	+/-Mouse & Rat liver and kidney S-9	10 mg/plate	neg.	Van Abbe et al. 1982
" "	TA1537	"	"	"	"
" "	TA1538	"	"	"	"
" "	TA98	"	"	"	"
" "	TA100	"	"	"	"
Syrian Hamster Embryo Cells - Enhanced viral transformation		Applied in sealed chamber	0.5 ml/chamber	pos.	Hatch et al., 1983
V79 cells - 8-azaguanine locus		Applied in flow through system, then sealed	3% in air	neg.	Sturrock, 1977
Human Lymphocyte - Sister Chromatid Exchange Induction		+/ - Rat Liver S-9	400 ug/plate	neg.	Kirkland et al., 1981
Human Lymphocyte Chromosome Damage		+/ - Rat Liver S-9	400 ug/plate	neg.	"
E. Coli WP2p and WP2uvr $A^{-}p$		+/ - Rat Liver S-9	10 mg/plate	neg.	"
Human Lymphocyte - Sister Chromatid Exchange in vitro			6 mg/ml	pos.	Morimoto & Koisumi, 1983
Mouse Bone Marrow - Sister Chromatid Exchange, in vivo			4 x 200 mg/kg/1	pos.	"
Rat Liver, DNA - binding in vivo				neg.	Diaz-Gomez & Castro, 1980

Table 4. (Continued) PAGE 2

Test System	Special Conditions	Highest Dose Tested	Result	Reference
Sprague Dawley Rat, DNA binding *in vivo*	Liver	N.A.	neg.	Pereira et al., 1982
	Kidney	N.A.	neg.	"
B6C3F1 mice, DNA binding, *in vivo*	Liver	N.A.	neg.	"
B6C3F1 mice, DNA binding, *in vivo*	Liver	N.A.	neg.	Reitz et al., 1982